

SEQUENCING AND COMPARATIVE ANALYSIS OF THE MITOCHONDRIAL CYTOCHROME B GENE IN THE  
MARION UPLANDS FLORIDA SAND SKINK (*PLESTIODON REYNOLDSI*)

by

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## Abstract

The Florida sand skink (*Plestiodon reynoldsi*)—a small (~10 cm) lizard endemic to the peninsula—is a ‘sand-swimming’ specialist restricted to Florida scrub habitat on the state’s central highland ridges. Florida scrub has been severely fragmented through urban growth and citrus farming, and less than 10% of this ecosystem remains. Given the skink’s limited geographic range and extensive population fragmentation, *P. reynoldsi* was listed as a federally threatened species in 1987. I surveyed skink populations from the Marion Uplands, where suitable lizard habitat is naturally (and has been historically) isolated from scrub on nearby Mt. Dora and Lake Wales ridges. I wanted to determine genetic relatedness of Marion Uplands skinks to those inhabiting these two ridges and hypothesized that Marion populations should be more similar genetically to those on the Mt. Dora ridge, given their geographic proximity. Mitochondrial DNA sequence analysis confirmed this hypothesis but also revealed unexpectedly high levels of genetic divergence between the Marion and Mt. Dora populations. Indeed, observed genetic divergence was comparable to that detected between Marion and Lake Wales populations.

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## Introduction

### *Plestiodon (Neoseps) reynoldsi* general background and biology

The Florida sand skink (*Plestiodon reynoldsi*), originally described as the monotypic (single species) genus *Neoseps*, is a small (~10 cm) burrowing lizard endemic to the Floridian peninsula. The skink is highly specialized to scrub habitat, using locomotion termed “sand-swimming” to move about. This locomotive form is permissible by several adaptations, including vestigial limbs, with one toe on the front limbs and two toes on the hind limbs, and a wedge-shaped head with a countersunk lower jaw (Richmond et al., 2009; Florida Fish and Wildlife Conservation Commission, 2016). These adaptations are highly specialized to allow the skink’s eel-like movement through loose sand.



**Fig. 1-2.** *Plestiodon reynoldsi*, depicting adaptations

### *Florida Ridges*

The sand skink is endemic to a restricted portion of Florida’s central highland ridges, specifically the Lake Wales ridge, Mt. Dora ridge, and the Marion Uplands. This habitat is characterized by scrub vegetation and sandy soil. *Plestiodon reynoldsi* is normally found just at the subsurface level of the sand, typically below foliage growth and debris (Pike et al., 2007). Skink dispersal is reliant upon well-drained, loosely packed soil, without dense root systems, as roots restrict movement (Pike et al., 2007).

Florida scrub and its endemic species, like *P. reynoldsi*, is at considerable risk due to human development. Urban growth and citrus farming have dramatically fragmented and reduced suitable

habitat for the skink, and less than 10% of Florida scrub remains (Branch et al., 2003; Richmond et al., 2009). In addition, fire suppression has led to the overgrowth of scrub plants and invasive species, restricting the skink's movability in the soil (Pike et al., 2007). Less severe risks to the skink's survival include road mortality and direct interactions with humans (Pike & Roznik, 2009). As the skinks occupy such a limited range, they are highly vulnerable to habitat disturbances (Richmond et al., 2009; Pike & Roznik, 2009). Smaller populations lose genetic variation much more quickly than larger ones, and for the skink, this risk is increased by the reduction and fragmentation (Richmond et al., 2009). Loss in genetic variability reduces population survivability, or fitness, as deleterious alleles are often propagated through inbreeding (Richmond et al., 2009). Due to habitat decline and possible restrictions in dispersal ability, the skink has been listed as "Threatened" by the U.S. Fish and Wildlife Service since 1987 (Florida Fish and Wildlife Conservation Commission, 2016). Overall, the skink is at a risk of extinction as a result of the loss of suitable habitat.



**Fig. 3-4.** Examples of scrub habitat. The skink would be found just below the sand, often below foliage or other cover.



**Fig. 5.** Tracks of *Plestiodon reynoldsi*

#### *Plestiodon reynoldsi* population genetics and phylogeography

Florida sand skink populations from the Mt. Dora and Lake Wales ridges have been analyzed in previous population genetics surveys. These studies revealed considerable genetic divergence between the Lake Wales and Mt. Dora ridges, indicating the onset of prolonged isolation prior to human development (Branch et al., 2003; Richmond et al., 2009). Natural divergence of *P. reynoldsi* may be a product of “vicariant events associated with the geology, oceanography, and climate of the Florida peninsula” (Branch et al., 2003). The central location of the Mt. Dora and Lake Wales ridges suggests that these ridges were not submerged during Plio-Pleistocene sea level fluctuations that led to peninsular fragmentation. The resulting geographic isolation is thought to have promoted speciation and endemism of Florida ridge biota. This view has been supported by phylogeographic structuring observed among evolutionary lineages in other species that inhabit the Florida peninsula (Branch et al., 2003).

#### *Marion Uplands population and focus of study*

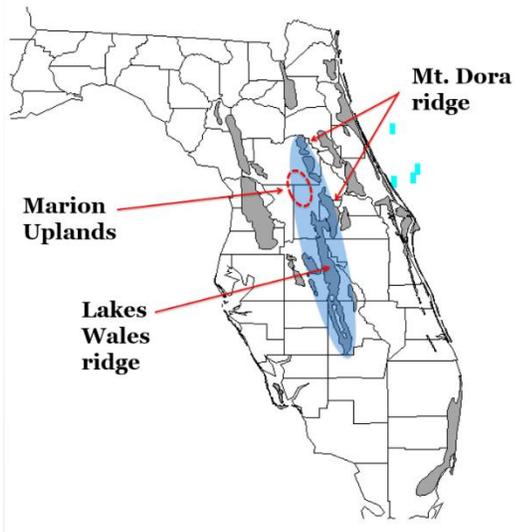
My study focused on skink populations from the Marion Uplands, which has not yet been studied. The Marion Uplands are located northwest of the Lake Wales ridge and runs along the upper westward border of the Mt. Dora ridge. I wanted to better understand the evolutionary relationships

between the Marion Uplands skink populations and those from major neighboring ridges, Mt. Dora and Lake Wales. A more complete understanding of the genetic relationships among these populations will assist in the planning and enforcement of effective management plans for *P. reynoldsi*.

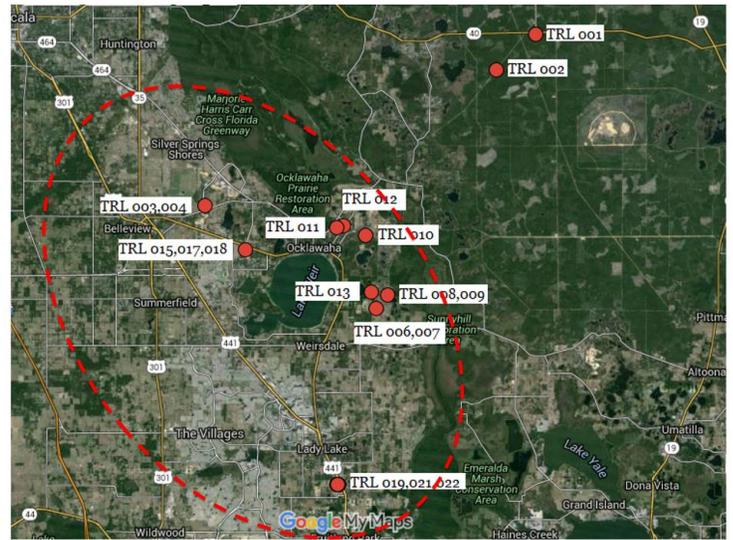
I hypothesized three possible results: 1) the Marion Uplands population would be more genetically similar to populations on the Mt. Dora ridge, as they are closest geographically; 2) Marion Uplands population may be more genetically similar to populations to the south on the Lake Wales Ridge; or 3) Marion Upland populations may exhibit significant genetic divergence from both the Lake Wales and Mt. Dora populations, given potential geographic barriers, such as the Ocklawaha River, which lies between the Marion Uplands and Mt. Dora ridge.

#### Methodology

I isolated genomic DNA from clipped tail tips (N = 23), provided by Paul Moler, a herpetologist with the Florida Game and Fish Commission. As the skinks are federally protected, tail tip collection allows the skinks to be processed and released at their capture sites (Fig.3). I opted to use the mitochondrial DNA gene cytochrome b (*cytb*), an informative genetic marker used in previous population genetic surveys of this species. I amplified a 600 base pair segment of the *cytb* gene via polymerase chain reaction, utilizing primers LGL765 (light strand, Bickham et al., 1995) and H15149 (heavy strand, Meyer et. al, 1990). I compared the Marion Uplands skink's *cytb* sequences with those published for the skinks from Mt. Dora and Lake Wales ridges, available on (and downloaded from) GenBank, and analyzed patterns of genetic relatedness among the Mt. Dora, Lakes Wales, and Marion Upland populations.



**Fig. 6.** Geographic range of *Plestiodon reynoldsi* imposed over Florida's major ridges.

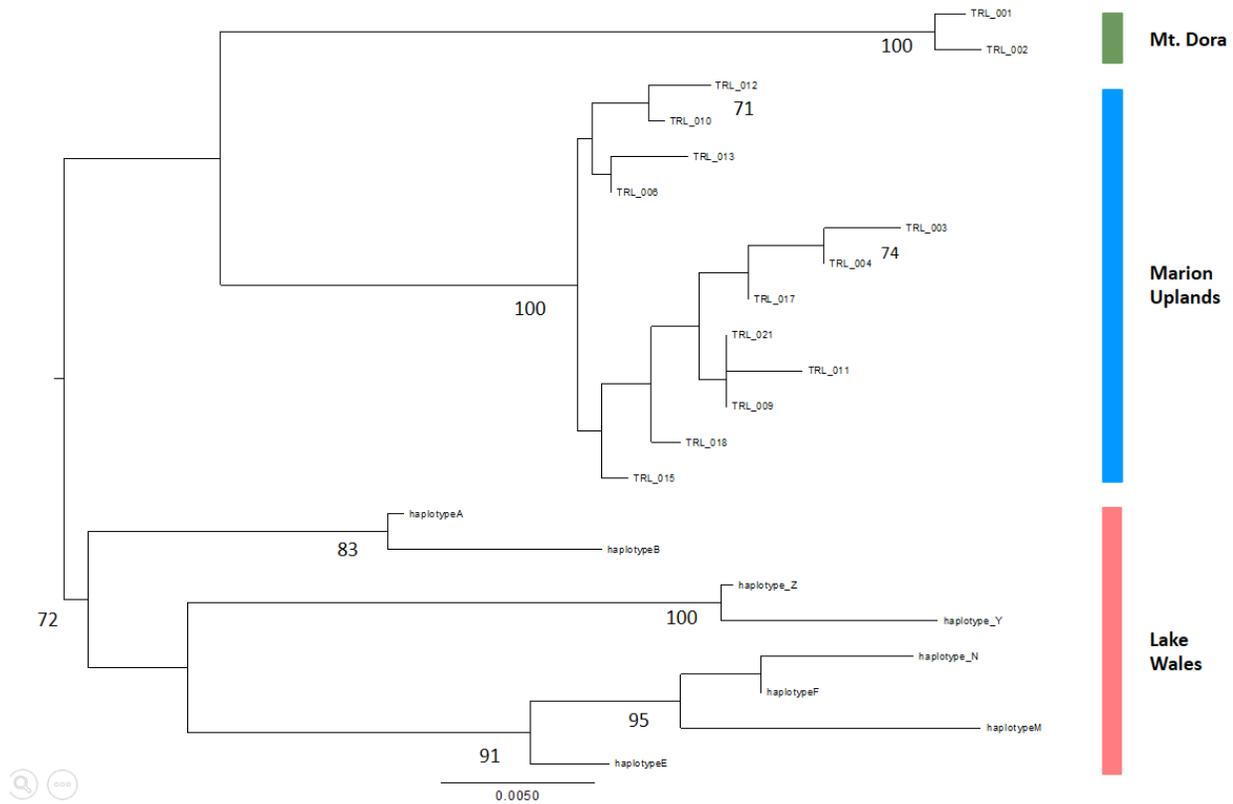


**Fig. 7.** Collection localities for skinks from the Marion Uplands (delimited by red oval). Sites 001 and 002 are on the Mt. Dora ridge.

## Results

I identified 11 *cytb* haplotypes among the Marion Uplands samples and compared them to haplotypes from the Lake Wales ridge. I assimilated these comparisons into two figures, a neighbor joining tree (Fig.8) and a median joining network (Fig.9).

A neighbor joining tree (Saitou and Nei, 1987) organized the selected populations by their evolutionary relationships. Bootstrapping assisted in determining the level of reliability for these relationships. Bootstrapping resamples individuals out of the phylogenetic tree to find if the characteristics that determine their relationships are consistent (Holmes, 2003). A value of 100 indicates that the same node occurs with every resampling; values below 70 are considered to be unreliable.

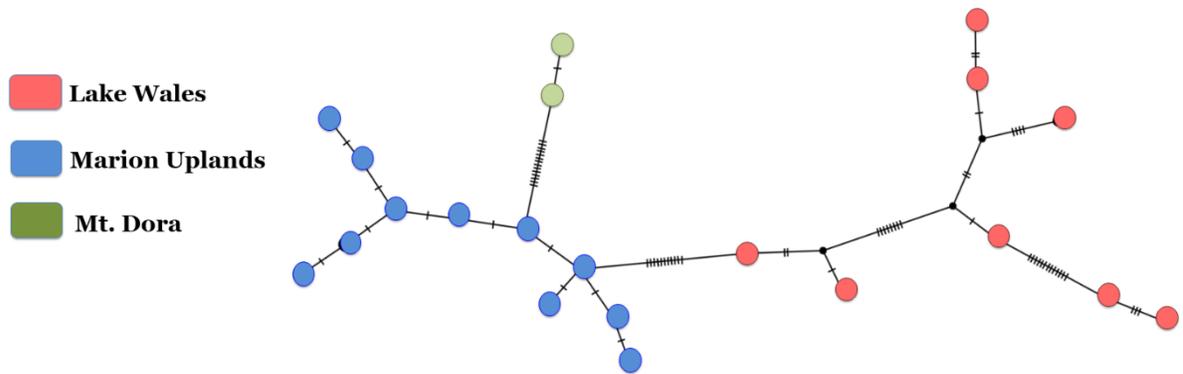


**Fig.8.** Neighbor joining tree depicting evolutionary relationships between skinks. Floating numbers represent bootstrap values; bootstraps below 70 are not reported as they are poorly supported.

A network analysis (median joining network) (Bandelt et al., 1999) linking Marion, Mt. Dora, and Lake Wales haplotypes supported the hypothesis that Marion and Mt. Dora populations are more closely related. To quantify genetic relatedness, I utilized a simple equation:

$$\frac{x}{400} \cdot 100$$

Value  $x$  is the number of nucleotide substitutions between a pair of samples. Cytochrome- $b$  strands of 400  $bp$  were utilized for comparison. Between the Marion Uplands and Mt. Dora populations, genetic divergence was approximately 3.5%. Values observed for Marion and Lake Wales haplotype comparisons ranged 4-5%. Thus, the *cytb* data on the Marion Uplands skinks revealed genetically distinct populations and contribute significantly to the overall evolutionary profile of this threatened species.



**Fig.9.** Network depicting relatedness among skink haplotypes. Hatch marks represent nucleotide substitutions.

### Discussion

My results were concurrent with findings from previous research. My hypothesis regarding closer relatedness between the Marion Uplands and Mt. Dora populations appeared to be affirmed (Fig.9), but divergence between the Marion Upland and Mt. Dora populations, at 3.5%, was higher than expected and is comparable to genetic divergence observed between the Mt. Dora and Lake Wales ridges (4-5%). A previous genetic comparison found genetic divergence between the most southern Lake Wales sample and northern Mt. Dora sample (therefore, the two samples from each ridge that were closest geographically) to be 3.2% (Branch et al., 2003). The most geographically distant samples diverged by 4.8%, and all other comparisons averaged 4.5% (Branch et al., 2003). In addition to the considerable genetic divergence, nodes defining relationships between Mt. Dora and Marion Uplands populations in the neighbor-joining tree (Fig.8) received little bootstrap support. Therefore, the Marion Uplands population may be equally divergent from the Mt. Dora and Lake Wales populations.

Collecting *cytb* data from more skinks would lead to more concise divisions, particularly from a wider range of localities. Many samples collected from the same or nearby regions shared the same haplotype. While it is not abnormal for identical haplotypes to occur within a such a restricted population, this phenomenon may be an indicator of losses in genetic diversity (Branch et. al, 2003).

Conserving threatened and endangered species is a challenging venture, particularly for narrowly endemic species in areas like central Florida. Often human growth exceeds the rate at which conservation efforts can be implemented, and legislation is slow and sometimes expensive, especially when it is forced to limit expansion of businesses and neighborhoods (Pike et al., 2008). Ultimately, my conclusions should contribute to the overall genetic profile of *P. reynoldsi*, and may assist in the development of comprehensive conservation strategies to preserve the skink. Conservation strategies will likely also be beneficial to other plant and animal endemics from the Florida peninsula. The sand skink's clearly distinct lineages make it ever more important to preserve these remaining populations (Pike and Roznik, 2009).

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